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RETINAL INFORMATION PROCESSING FOR MINIMUM LASER LESION DETECTION  
AND CUMULATIVE DAMAGE

FINAL REPORT

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13. ABSTRACT (Maximum 200 words)  Minimum ophthalmoscopically visual lesions are not perceived very well by the injured person either from clinical experience or other forms of testing, certainly not with the detail that retinal images of the same size are seen. Previous experiments have suggested a model for retinal organization suitable for small detail detection but which will not detect retinal small lesions very well. Smaller lesions of a sub-threshold nature can cause histological damage but are not visible ophthalmoscopically, nor can they be demonstrated to cause a loss of function. A series of animal experiments has been carried out to test the electrophysiological responses of retinal ganglion cells with sufficient resolution to detect threshold laser exposures with possible deficits in perceiving moving spot/edges and other optical discontinuities. The results are accurate with high resolution and the results should be correlated with supra-threshold exposures of a level to cause sub-retinal hemorrhages and/or vitreous hemorrhages. Both qualitative and quantitative comparisons are needed between the kind of damage caused by sub-threshold, barely threshold, and markedly supra-threshold lesions on this aspect of retinal organization.				
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## I. Introduction

The purpose of this program has been to validate a method which gives sufficient resolution to assess small changes in information processing capacity in the retina, particularly relating to visual acuity following injury to the photoreceptor layers such as detachment from the pigment epithelium by hemorrhage and any subsequent therapy such as surgical removal of the subretinal hemorrhage and chemical treatment to dissolve blood clots. All types of therapeutic intervention as well as the hemorrhage itself can be expected to destroy some photoreceptors and derange others from their specialized orderly arrangement so that the fine details of the structure of the retina may be disturbed. It is important to understand how much of an effect photoreceptor death as well as the disturbance of the precise anatomical pattern by the development of scar tissue or glial infiltration has on the functional integrity of the information handling in the retina. This is particularly important when the anatomical disturbances are small and in the central portion of the retina, the macula and especially the fovea. This is the region where information processing is most important, as it must make up for the poor optical quality of the retinal image. Overall, the measurement problem is to determine whether restoration to a 20/20 or 20/15 visual acuity performance level can be achieved in a healthy retina which has been subjected to subretinal hemorrhage and subsequent therapy.

In this program, we have studied in detail the particular part of retinal information processing upon which the highest degree of visual acuity depends. Visual acuity depends upon the transient interaction between the center and surround input processes to the ganglion cell receptive field. Our work has indicated that the type of information processing used by the fovea and the rest of the retina is not significantly degraded by destruction or derangement of a small number of the photoreceptors if they are not sufficient to cause a scotoma. In a retina with only a few missing cones, acuity performance can be achieved almost as well as in the normal state. That is, if some receptors are killed and others are tilted or moved slightly, high visual acuity should be still possible.

In our program, we have attempted to develop tests that can be made on a routine basis on animals that have had retinal injuries treated or untreated to document any changes relative to the normal responses. Other programs have shown that the blood clot formed by subretinal hemorrhage can be dissolved and removed with apparently good approximation of the retina to the underlying pigment epithelium, but there presently is no data to show how post-treatment visual performance compares with pre-injury levels, particularly with regard to the tasks requiring the highest visual acuity, including hyperacuity.

## II. Background

In order to understand the types of tests on retinal ganglion cells necessary to document the performance relation to a high degree of visual acuity, some review of the details of the visual system is required.

## A. Visual System Performance

An examination of the characteristics of the human visual system shows that it has considerably better performance than any artificial detector array has yet achieved. Histological investigations of the anatomy of the photoreceptor layer and data from optical measurements (Gubisch, 1967; Snyder and Miller, 1977) of the characteristics of the retinal image (point-spread, line-spread functions) indicate that the human visual system can detect separate sources whose angular subtenses are much less than the width of a single receptor (Westheimer, 1977). This is an apparent violation of the first order Rayleigh criterion for the lower limit of resolution of a detector array. Where the illuminated and unilluminated portions of the detector array image form the basis for detectability, the first order Rayleigh criterion requires that a detector array has, at least, one unilluminated detector between two illuminated ones to indicate two separate sources. When consideration is given to other relevant optical factors as listed below, this analysis of the threshold performance of the human visual system seems even more puzzling:

1. The photoreceptor detectors in the retinal array are similar in size to the wavelength of light detected.
2. At the limits of perceptual resolution, the focal plane on the retina has rather fuzzy diffraction-limited images (LeGrand, 1967).
3. Visual performance is as good in white light as in monochromatic, although the retinal image is much better in monochromatic (LeGrand, 1967).
4. Retinal image movement due to head and body tremor (and to some less important extent, small amplitude eye rotation) is present during the known integration time for the optimum detection of the image (Bengi and Thomas, 1973; van Buskirk, et al, 1966; and Ditchburn, 1973).

The material on visual acuity has been reviewed in detail by Gubisch (1977), Ditchburn (1973), LeGrand (1967). Wolbarsht et al (1985) also discussed the possible beneficial visual function of the small retinal image movements.

## B. Visual System Models

Prior models of visual system information processing have, at least implicitly, been based on point-to-point representation of the receptor array in the cortex. Although anatomical and neurophysiological studies for some time have suggested that this view, as originally formulated by Helmholtz (1924), is too simplistic; nevertheless, no model of the performance limit of the visual system has been based on other than an analysis of the match between the diffraction-limited-retinal image and the receptor array. The extensive interconnections which have been demonstrated between the retinal neurons processing the information gathered by the detectors (Kolb, 1977), as well as the limited number of fibers in the optic nerve (always less than the number of detectors) have either been ignored [For example, Polyak (1941) and others have suggested a direct connection between the detectors and the cortex in

the area of best vision, the fovea.], or the view has been taken that the system achieves its high performance by pattern recognition. That is, a system of guessing is assumed in which the higher visual centers construct a probable source that might have caused the degraded pattern represented at the cortical level.

For some time, evidence has been accumulating about the nature of the information processing in the retina. Some recent experiments show a radically different type of retinal information handling that suggests a model which seems to avoid the limitations discussed above (Wolbarsht, et al, 1985). The model derived from these experiments is based on several aspects of the information processing in the retina which suggest that it is not pattern recognition but rather pre-processing of the neural information which sets the limit to the detection performance of the system. Also, it is this information processing which extends the visual performance beyond the simple (or first order) Rayleigh Criterion a limit supposedly imposed by simple optical considerations of the retinal image.

For purposes of detecting small lesions in the retina, the present model for information handling in the visual system can be best thought of as using a discontinuity detector operating within a color framework to give a highly nonlinear representation of edges (discontinuities) without color significance in a highly compressed gray scale. The total visual perception includes the addition of colors whose intensity follows an almost linear scale around ambient light levels and which fill in the spaces between the optical discontinuities. This color intensity is represented on a linear scale by using feedback to adjust the midpoint of the intensity (gray) scale to the ambient lighting level. The present model of the visual system also suggests that there is compression of size representation. That is, small objects have a certain minimum representation, and there may be fewer locations (pixels) in the display array than in the detector array.

For modeling purposes, the most convenient place to represent the coded information from the detector array in the retina is as it leaves the retina in the optic nerve. The individual optic nerve fibers are the output of the retinal ganglion cells which are the third stage of computation in the retina. Further information processing is performed in the lateral geniculate, but this can be considered part of the decoding process.

The details of the human visual system which are important in the present program are as follows.

1. No cone (or rod) in the retina has a unique neural connection (transmission line) to a specific neuron in the cortical representation of visual space.

2. The visual system in common with most, if not all, other sensory and motor systems in animals is analogue in character for all processing and transfer of information. The nerve impulses, popularly viewed as digital information, are really pulsed-coded analogue data. The impulse spacing, or instantaneous frequency, and the bunching patterns are not digital but analogue information for the next stage. In general, the impulse patterns are translated into decremental slow potentials and

summed with incoming information from other transmission lines to form the basis for the next stage of computation.

### C. Retinal Ganglion Cell Receptive Fields

Each computing unit in the retina, including the cones and rods, has a positive and negative output to succeeding computational stages of neural units, possibly through interneurons. The information from each unit also has a lateral spread in passing to the next stage. Thus, by the time the third level of units, the retinal ganglion cells, is reached, a large area of cones in the retina is contributing information. These spheres of influence at the ganglion cell level are termed receptive fields, and in adjacent ganglion cells, they have a large amount of overlap. The retinal ganglion cell receptive fields have a complicated structure whose spatial sensitivity transfer functions can be represented in profile as shown in Fig. 1. These receptive fields can be represented as the sum of two Gaussian curves, one representing the positive input to the ganglion cell, the other representing the negative input. As the input Gaussians are not the same width, the summed output for steady state for input from the two Gaussians is represented by triphasic curve often termed "the Mexican hat" or sombrero.

The use of the triphasic curve (or Mexican hat) in Fig. 1 as a substitute for a simple step function profile allows both integrating and correlation functions to be incorporated in the computations for edge detection and localization. Nevertheless, this type of information processing is not the whole story as it is only a more sophisticated model of the point-to-point representation of the detector array. In this type of representation, the location of any discontinuity or edge in the retinal image still involves finding the ganglion cell whose receptive field center coincides with the cone indicating the location of the discontinuity. Thus, acuity and small scotoma detection would depend upon the identification of a specific ganglion cell with each photoreceptor, a one to one ratio between ganglion cells and detectors. As this is contrary to both the anatomy and physiology of the retina, if not all higher visual centers also, some other formulation must be sought.

### D. Retinal Image Movement, Mechanical Tremor

Another and perhaps the most important point to consider in any model is ocular tremor, the frequent small amplitude ( $< 15'$  of arc) random direction motions of the retinal image due to mechanical tremor of the head and body, and, to a probably negligible extent, eye rotation (Bengi and Thomas, 1973 and van Buskirk *et al*, 1966). This tremor is due to mechanical vibration of the eyes, head and body at their various resonant frequencies and is driven by breathing and heart motions when the posture is stabilized for best vision fixation. This tremor converts the detection of visual information into a dynamic process. Whatever may be the source of this small amplitude retinal image motion, the result is a semi-periodic movement, random in direction, about a common location. Large (saccadic) eye movements are too infrequent to affect acuity and are omitted in the present model. The frequency of the tremor (10 to 90 Hz) is well within the neural integration time under ordinary ambient lighting conditions. Thus, the transient response of

the visual system to such small amplitude image motions on the retinal detector array must be considered as part of the ganglion cell output during edge detection.

We consider that this very small amplitude tremor in conjunction with the retinal organization can emphasize image "edges" or optical discontinuities in the following way. The actual ganglion cell responses to small retinal image motions are shown in Fig. 2. In this case, the interplay between the transient positive and negative inputs to the ganglion cell receptive field produces a cup-shaped response profile rather than the "Mexican hat" produced by the steady state inputs. The transient response from the image movement is minimum in the center, with a bimodal maximum response in the regions where the positive and negative inputs are approximately equal. This response to motion is a burst of transient high instantaneous pulse repetition frequency. This transient response rides on the background steady state pulse frequency response representing the average intensity of the light on the center of the receptive field. In the neural analogue system, both types of information can be carried along the same ganglion cell axon, as they have quite different pulse repetition frequency domains.

#### E. Visual System Information Processing With Tremor Contributions

The portion of the information carried by the transient high pulse repetition frequency response resulting from motion can be used to localize discontinuities in the following manner. The maximum output from a single ganglion cell in response to a small displacement of a discontinuity within its receptive field is eccentric to the center of its receptive field. Thus, the motion of an optical discontinuity imaged at a particular retinal location would give a maximum signal in the ganglion cells located eccentrically, but in a circular fashion, about the locus of the motion in the detector array as shown in Fig. 3. This flow of information to the retinal ganglion cell converts the problem of locating the discontinuity on the array of retinal cones from one of finding a single ganglion cell whose output is different from the others to one of finding the ring or cluster of ganglion cells whose output is different from adjacent clusters. It can be compared to the information processing involved in finding the hole in the doughnut by locating the doughnut. That is, subsequent stages of information processing can localize the discontinuity as the weighted average of a cluster of ganglion cells whose center of gravity is the ganglion cell (or cells) in the center with a minimal response. Three dimensional views of both the static and transient receptive fields are shown in Fig. 4.

The above analysis, which shows how the retina has incorporated small amplitude retinal image motion to achieve higher resolution, is in contrast to previous models which have considered such motion as degrading the achievable acuity. For example, Wulich and Kopeika (1987) suggest that all motion blurs the image, and that, therefore, the probability of achieving the maximum resolution rises as relative exposure time is decreased. On the other hand, the present model suggests that the longer that fixation is achieved to allow integration between large image excursions due to saccades, the better the resolution.

Obviously, in this system, the precise location of any single detector cannot be represented in the output. Only the presence of the discontinuity itself can be represented. Indeed, since motion is involved in the detection, the exact retinal location can not be specified. In fact, the overall performance of this system can be considered to be represented by a sort of "Heisenberg Uncertainty Principle" applied to vision in which, at threshold, the exact location of a discontinuity on the retinal detector array is reciprocally related to the certainty of its presence. However, the location of an edge with respect to any other edge in the overall retinal image would be precisely given, almost it seems as a function of light intensity. With this type of information processing, small discontinuities can be detected more reliably, yet with less certainty about their image location on the retina. Since the presence of a discontinuity is represented only as the center of gravity of a cluster of ganglion cell outputs, accurate analysis of absolute size may be lost for very small discontinuities. However, relative judgments of size between two small discontinuities of similar contrast may still be given with certainty and accuracy.

The most interesting consequence of this analysis is the lack of correspondence between the perceived object and its location on any particular cone. Thus, if a single cone (or small group of cones) dies or is not functioning, the cortical representation of the retinal image is virtually unchanged. This almost certainly accounts for the lack of small scotomas due to single missing cones or other neurons in the retina.

The neural impulse information coming from the ganglion cells is almost certainly analyzed by splitting it into high and low instantaneous impulse frequency components. As described above, the high frequency (transient) component locates a discontinuity eccentric to the receptive field center. The steady state or low frequency component would be representative of the average stimulus on the center of the receptive field and, thus, will give intensity information. As each cone has its individual wavelength sensitivity, color discrimination can be achieved by a comparison of the low frequency (steady state) responses from the various ganglion cells with different types of central cone connections. This analysis would have to be carried on by a pathway substantially independent of the one for cluster analysis and edge detection.

The actual ganglion cells found in the retina have both positive and negative inputs from cones and interneurons (bipolars, amacrine) to the central and peripheral portions of the receptive field, as explained in the legend to Fig. 1. Since the vast majority of the cones are summed equally for detection of discontinuity information, the presence of color detection does not seriously degrade the performance of the system for discontinuity detection. Different channels connect the detectors to the ganglion cells for discontinuity detection and for color and intensity information. The gain of each neural channel can be adjusted separately before combination at the ganglion cells level as a function of ambient light conditions. In this way, the gray scale representation of the system can be fairly linear over a factor of about 100 with reference to the ambient lighting level. The setting for the ambient intensity can itself shift over the entire intensity range of human vision which has a sensitivity range of a million or more.



#### F. Detection of Minimal Retinal Damage Sites

The main point of this program is to detect or represent reliably small areas of damage in the retina such as the loss of a single cone or a small group in so far as they affect retinal function. The preceding discussion shows how the retinal organization allows a high degree of visual acuity without, however, being limited by the size and exact layout of the cone mosaic. Although loss of a single cone has little effect, the continued loss of cones will degrade the ultimate resolution attained by such a system. The loss of cones will blur the center surround organization of the ganglion cell receptive field, and the ganglion cell transient response will probably show the earliest and most pronounced effects. Our theory suggests that the cup shape in Figs. 2 and 4 will lose its central dip and become dome-like and flatten considerably. This diminution of the transient response will blur edges and make the neural input from this retinal area similar to that from a stabilized image in which this is no transient response. The ultimate result of such damage would be a visual scotoma, but visual acuity in this region should be degraded first. If this loss of receptors is due to the delayed effect of intraocular hemorrhage, there is the possibility that prompt removal of the blood may ameliorate much if not all of the subsequent damage.

An analysis of this model shows that the loss of single detectors do not produce blind spots or miniature scotomas. This can be compared to the image transmitted by a coherent optical fiber bundle. A lost fiber would produce a black spot, but jiggling or otherwise moving the fiber bundle would get rid of the black spot in what is usually thought of as a picket fence view. Even in a normal retina, cones die, and although they are not replaced, visual acuity in the central portion of the retina, the fovea, changes very little. In fact, the loss or faulty performance of a single detector only causes a simple addition of a little bit more noise to the visual signal, but the overall visual transfer function for contrast sensitivity in that area of the retina would change very slightly. Indeed, the contrast sensitivity function is found to be lowered with age on a continuous basis which is probably due to both the loss of photoreceptors and of neurons throughout the visual system the increased light scattering in the optical portion.

As discussed in section II, D above, the small amplitude, random retinal image movement which we term ocular tremor can be caused by both the mechanical vibration of the head and eye due to breathing, heart motion, and muscular activity and the random movements in the extraocular muscles often called "small eye movements". These mechanical displacements from breathing and the heart beating cause the more or less rigid head, eyes, and torso to vibrate at their resonant frequencies. The resonant frequencies of the head and eyes seem to be in the 20/40 Hz region with some sizeable contributions in the 10 -20 Hz range and even some minor peaks at higher frequencies near 90 Hz.

Experimental attempts to produce stabilized retinal image usually eliminate all retinal image shifts due to eyeball rotation, such as "small eye movements", from changes in extraocular muscular tension (Ditchburn, 1973). These experimental conditions sometimes also decrease the amplitude of the mechanical vibrations, but this component of retinal

image shift can only with great difficulty be completely stabilized. However, use of a bite bar and other mechanical stabilization techniques can produce an image stable enough on the retina so that the transient component of the ganglion cell response disappears. If this transient disappears, the classical signs of the stabilized image appear. The borders in the central portion of visual space disappear, and this is followed gradually by a disappearance of all borders after which the colors run together, and the visual scene becomes a uniform featureless gray.

It should be noted here that eyes that develop some types of pathology or have developmental errors (ocular albinism) which result in nonfunction of the fovea and portions of the central macula leave the retina with only large receptive field ganglion cells. In these cases, the ordinary mechanical induced tremor motions are not large enough to produce significant transient responses in the large receptive fields in the remaining portions of the retina. The eye then adopts a large amplitude rapid motion of its own, nystagmus, to improve visual acuity. It is, thus, not surprising that when this nystagmus is stopped, visual acuity deteriorates.

### III. Experimental Procedures

External recordings from retinal ganglion cell bodies and nerve fibers at the optic disk have been used to monitor the sensitivity of the retinal ganglion cells for minimally moving spots and edges and to document which exposure parameters will distort or lessen acuity information as it leaves through the optic nerve. Intraocular metal microelectrodes were used to monitor the nervous activity in the retinal ganglion cells of cats. The structure of the receptive field of the ganglion cell was mapped, and the responses following the test laser exposures were determined. Changes in impulse frequency response to a stimulus following a laser exposure may be used as a criterion for destruction of the part of the pathway for information flow and correlated with the responses previous to the time of the exposure.

#### A. Optical Stimulation

Optical stimulation is provided by a Maxwellian view system adopted from a two independent channel system similar to that described by Wagner et al., (1960), and modified with interference filters substituted for monochromator, as described in Crocker, et al., (1980). The two channels can have either interference filters or broad band gelatin filters (Wratten type, Eastman Kodak Company, Rochester, NY) for chromatic adaptation. The beams from the two channels can be combined so that they will pass as a single beam through a flat front face corneal contact lens and the center of the fully dilated pupil. In this configuration, the beam forms a focused, demagnified (10:1) image of the optical stimulator apertures on the retina. Each of these co-extensive beams covers 20  $\mu\text{m}$  of the retina.

A good description of the sensitivity of the selected retinal ganglion cell before and after subthreshold laser exposure ensures a good knowledge of the type of response, if any, that would be recorded immediately in response to a suprathreshold laser exposure. This

documentation enables any optic nerve response to be identified as to what message a supra-injury-threshold laser stimulus would have given to the higher visual centers, and therefore, what kind of perception might have occurred or whether the receptive field indicates any pathological changes in the retina.

The Maxwellian type of view stimulus allowed sufficient intensity of chromatic narrow band stimulating light to be delivered to the retina to characterize the performance of any selected ganglion cells with a high degree of precision.

The field aperture of the optical stimulator is focused on the retina and can be as small as 20  $\mu\text{m}$ . Previous work has indicated that this is a highly reliable figure as it has been compared with known size of probes inserted into the eye and measured at the same place as the stimulus spot on the retina. The stimulus beam is approximately normal to the retina, eliminating any change in stimulus response curves from the Stiles-Crawford effect.

In order to achieve the Maxwellian view for the stimulus, a flat face contact (gonial) lens is used on the cornea to eliminate that refracting surface, and assist in visualizing the posterior pole of the eye for long periods of time. Various configurations of this contact lens have been tried, including a rigid oxygen non-permeable lens or a soft contact lens which is highly oxygen permeable. Flowing well oxygenated solutions around the edges of a hard contact lens has, in the past, ensured that the cornea remains clear during the course of the experiment, and this technique was used in the present program.

Laser beams for lesion formation during the course of the experiment were introduced through a third channel and located/focused with a coaxial, low power (0.5 mW) helium neon laser indicator beam attenuated to a minimum stimulus level. The basic Nd-glass (also ruby) laser system has a 0.5 cm x 7.5 cm neodymium glass rod and can be Q-switched. It has an initial wavelength of 1,060 nm, and gives 530 nm with a KDP doubling crystal. The beam is only 5 mm in diameter at the cornea, and thus, passes completely through a dilated pupil. Other types of longer term laser exposures can be accomplished with CW lasers before the experiment with various auxiliary equipment, such as an argon photocoagulator, or during the experiment through the third channel.

Absolute calibration was carried out for the stimulator and the laser equipment. All calibrations were compared against standard secondary sources whose calibrations can be traced to the National Bureau of Standards.

## B. Electrophysiological Techniques

Extracellular recordings were made from retinal ganglion cells and optic nerve fibers from the intact eyes of adult cats. The amplified signal responses from single isolated cells were used. The electrophysiological recording equipment has been described previously in detail (Crocker *et al.*, 1980), but was modified by the use of a low noise preamplifier/amplifier system designed by Prof. E. F. MacNichol, Jr. and

constructed at the NIH. The excellent recording characteristic of this system required no additional filters or other circuitry for impulse recognition or separation of signals from co-recorded neurons.

Tungsten or stainless steel wire, electrolytically sharpened electrodes, plastic insulated to the tip, similar to those described by Levick (1972) were used in conjunction with a FET amplifier input stage. These electrodes were procured commercially from AM Systems, Inc., 122075 St. SW, Everett, WA 98203 (Cat. #5710) with a tip resistance of 5-12 megohm. The higher values gave better unit isolation, but, overall, had a lower success rate for recording rates per retinal penetration. The electrode and carrier are inserted into the eye through the region of the pars plana, as in conventional vitrectomy surgery. The electrode is then placed against the retina under visual observation through the anterior part of the eye. When the electrode is positioned in front of the selected part of the retina, it is moved forward until it just penetrates the internal limiting lamina. Most recordings were made from a single ganglion cell body or its nearby axon. In this type of recording, most of the electrode positions are at or near the center of the receptive field of the ganglion cell. As there can be mechanical motions of the electrode due to laser exposure, recordings were also be made from the ganglion cell axons where they enter the optic disk. This was done in the same fashion by advancing the electrode to the rim of the disk. Previous experiments have shown the recording made at the disk are from the same kind of cells as are found by cell body or nearby axon recording. The recordings at the optic disk are stable and seem capable of continuing from the same neuron after laser exposure to a portion of the retina in the receptive field of that ganglion cell.

It is, however, more difficult to find optic nerve fibers at the disk whose receptive fields are within the range of the optical stimulator at the settings when the cell is acquired. Also, recording times are much shorter, although some recordings have be made with a duration of several hours. The technique to achieve these long lasting recordings from the disk needs to be optimized in future programs so that the data for markedly suprathreshold laser exposures can be more reliably collected from this type of recording.

All data points were determined to show the initial sensitivity of the ganglion and its other parameters by a constant response technique. This is in order to ensure that we can interpret the given response from the ganglion cell to laser exposure. The ON/OFF response characteristics are documented as well as the chromatic responses of the cell relative to the location in receptive field for both center and surround. All types of cone contributions will be identified in order to determine, if possible, which of the cone types gives rise to the acuity response for minimal sized retinal images and their motions.

### C. Animal Procedures and Surgery

Intraperitoneal pentobarbital is used to induce and maintain anesthesia during initial surgery for leg vein cannula insertion. All subsequent surgery for insertion of a tracheal cannula and stabilization of the eye on a ring sutured to the sclera between the extraocular

muscles is performed with pentobarbital administered i.v. In addition, there is subcutaneous injection of a local anesthetic on all incision sites before surgery. During the recording portion of the experiment, the i.v. pentobarbital is replaced by i.v. Ketalar, and a general inhalation anesthesia, 70% nitrous oxide/30% oxygen mixture, is used. The inspired  $pCO_2$  is monitored continuously and kept at approximately 4.7%. Gallamine triethiodide, dextrose, and saline infusion are used i.v. during the recording session to aid in stabilizing the animal and fixing the eye.

Although nitrous oxide, even at high partial pressures, does not produce complete surgical anesthesia (Brown et al, 1920, Venes et al, 1971), it has been shown that 70% nitrous oxide with 30% oxygen produces a high degree of sedation and analgesia in both cats and monkeys. It is an adequate anesthetic where only mildly noxious stimulants are present; for example, the direct electrical stimulation of peripheral nerves at frequencies of up to 3 Hz or foot pad shocks. (Venes et al, 1971). In the experiments in this program, the animals are under deep anesthesia at levels sufficient to terminate spontaneous respiration and require artificial ventilation, and, as mentioned above, i.v. Ketalar is also used at the rate of 0.5 mg/kg.

The insertion of the recording microelectrodes through the pars plana involves no pain. It is similar to operations often carried on in humans with only local anesthetic. We monitor the heart rate continuously and increase the level of anesthetic when changes in heart rate are detected that could be associated with pain perception. Nitrous oxide will be used because it has been shown to have only slight effects on evoked CNS responses, as compared to the strong depression induced by other volatile anesthetics and barbiturates (Van Norren and Padmos, 1977). A depressant action in the retina has been seen with some of the other anesthetics as well. Obviously, it is important to minimize drug effects on the CNS when studying the activity of the visual system.

The gallamine triethiodide is not required to relax the animal, but it will assist in establishing the high degree of eye immobility required for single cell retinal recordings (Enroth-Cugell and Robson, 1966). It has also been established that gallamine triethiodide has no effect on retinal ganglion cell responses in cats and monkeys (Enroth-Cugell and Pinto, 1970). We use nitrous oxide and gallamine triethiodide because of these considerations.

The normal body temperature is maintained at all times with a heating pad. Cats can be maintained in satisfactory physical condition this way for 24-48 hours, although the experimental procedures during this series of experiments were never more than 8 hours. The iris is dilated and accommodation relaxed with several doses of Duke mix (10% phenylephrine 0.5% mydriacyl, 1:1) or atropine sulfate applied approximately every hour.

All animals involved in this study have been maintained and used in accordance with the Animal Welfare Act of 1970 and subsequent amendments. Further guidelines were the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources (revised in 1985) and the National Research National Research Council (1966) and recommendations of the Duke Animal Care Committee.

#### IV. Progress During the Experimental Period of the Contract.

All of the necessary animal life support and electronic equipment for the experiment were put into running order and checked, the optical system calibrated, and the laser exposures have been measured to make the results comparable with other studies on retinal injury thresholds.

After preliminary experiments on model neuronal systems had been accomplished in order to check that all equipment is worked and that personnel had been trained to carry on the procedures properly, data were gathered during the initial period comparing the new experimental recordings with previous data to insure continuity with previous programs.

The purpose of the research is to determine the smallest movement within the retinal ganglion cell receptive field that can be detected, and the optimum velocity for that movement. It should be emphasized that although these retinal velocities are very high compared to those from normal eye saccades, they represent only extremely small retinal image movements or displacements. Moreover, they are at extremely high repetition frequencies, because they are mainly due to the mechanical movement of the retina itself as a part of the eyes and head which move as a unit with respect to the visual image.

After the first successful animal recording experiments had been achieved, base line information was collected on normal ganglion cell types. Experiments on hemorrhage initiation were tried at the end of each recording session by using a Q-switched Nd:YAG, but reliable peripheral hemorrhages which spread into the area centralis were not yet achieved reliably. At first, the experiments were limited to 3 hours of recording after which the cat's physical condition deteriorated rapidly. This hampered collection of data in the numbers of ganglion cells per retina we had hoped to achieve. The initial experiments were too short to get more than just one or two cells per eye. As it was necessary to define the spectral sensitivity curves for each cat, this did not allow much time for refinement of the responses to small motions and the definition of the high visual acuity. It was difficult to characterize the angular velocity for sufficient ganglion cells to understand any variations.

As the program proceeded, the rate of successful recordings for electrode penetration per eye increased greatly, as did the number of successful recordings in selected retinal locations. An electrode penetration is defined as an insertion of the electrode into the vitreous space, and placement in different retinal locations for each recording. As our goal was to achieve recordings from specified places within the retina adjacent to selected laser lesions or along the borders of subretinal hemorrhage sites, a high success rate was reached in order to collect sufficient data for analysis of retinal injury. This selection of the retinal location is most needed when any medical or surgical therapy has been attempted to mitigate or remove a subretinal hemorrhage or retinal detachment.

Midway through the contract period, the success rate for a selected site was about 40%. Although this is more necessary to be a usable level

for documentation in eyes which have not been treated, we had as our target to achieve at least 80% for testing eyes following any post-laser therapy or for tracing the degeneration or failure of neurons following laser exposure without subsequent therapy.

As the research progressed, significant progress was made in cat survival time and in increasing the rate of successful recordings for electrode penetration per eye.

Fig. 5 is a typical plot of the location of different types of ganglion cell responses in the central retinal area showing the ganglion cell types and success rate found by a given electrode. The recording situations were short and well stabilized, with the data easily and rapidly collected.

Many different ganglion cell types were found in portions of the area centralis, most of which fell into the X, Y, and W cell groups. The area centralis in the cat corresponds to the macular area including the fovea in the human retina. The largest number of cells were found in the X (sustained) category, while the Y (transient) had a smaller number.

Both X and Y cells had depressed responses in their central fields for small movements, as is shown from the data on minute movement of minimum sized spots under continuous illumination.

Since W cells were found only rarely, they probably can be ignored in any neurophysiological test for retinal acuity or other critical functions probably within the macula, and certainly within the fovea. Moreover, we are more interested in those cells which ultimately project to the cortex, and as the W group of cells go to the superior colliculus to activate the visual reflexes, they do not contribute to visual acuity.

At the end of the research period, the rate of successful recordings per electrode penetration has been sufficiently high enough (approximately 75%) to make it possible yet to record from a given animal at a given place in the retina. Almost every retina can be recorded from, and almost all approaches with the electrodes are successful. At the end of the contract, the success rate probably ranged about 75%, which gives sufficient information for accumulation but was a slightly lower value than we had wished to accomplish finally.

Our main objective, to achieve successful recordings and to expand our data bank in order to speed up identification of ganglion cells, has been achieved.

A detailed examination of the experiments to date illustrates the present protocol and shows the typical findings.

In the experiment shown in Fig. 6, the ganglion cell was isolated and identified as a single unit approximately 2 hours after the animal preparation began. Then, a preliminary run through of the spectrum showed the cone types connected to the ganglion cell by using both a central test spot and an annular surround, so the areal extent of the cones connected to the ganglion cells could be identified. This

particular retinal ganglion cell is an X type that had 540 nm (red) and 500 nm (green) cone types connected to the center, and in the periphery, a 540 nm cone system as is usual in an antagonistic fashion.

The X category means phasic or sustained response type, that is, the ganglion cell, is not very sensitive to gradual diminution of the total light intensity over the receptive field. The sensitivity to small movements of portions of the receptive field however, are easily detected. This sensitivity is shown in the second plot as the motion of a small spot within the receptive field. In this portion of the experiment, the light was left on continuously, and the spot rapidly moved a predetermined amount. A 75  $\mu$ m spot as projected in the plane of the retina was moved until it was just touching itself in a 1 ms duration motion by an optical lever in the optical simulator. This represents an angular speed of over 250 degrees per second, (1 ms on the retina to move 75  $\mu$ m). In any case, with this motion, the sensitivity obviously was much lower in the field center than it was somewhat eccentric or radial to this site. Then again, the sensitivity decreased rapidly further away from the center.

This entire portion of the experiment took about 1.5 hours to achieve. The characterization of the cell has become more rapid, and we need to perform less of an identification protocol and can do it more rapidly. At the time this experiment was performed, we had tried several electrode tracks in this eye before this particular cell was found, which was the first successful one in this eye. Approximately 10 incomplete recordings (insufficient recording time) and more than 15 probes in which no identifiable single units were needed before this cell was found. The 10 identifiable units that were achieved held onto for varying amounts of times, 3 mins., 8 mins., 17 mins., 14 mins., and 36 mins.. It should be noted that 36 mins. should be long enough to make a positive identification in the future, but the plan is to do it in still less time as the protocol becomes simpler. In future experiments, we needed only a 5 min. duration for success. This gave us an enormous increase in the number of cells that can be used. Once this goal was achieved, we were ready to try to document loss of function and recovery in animals that have had test hemorrhages with possible visual decrements from other ARMDC programs.

Some diversity of the spectral response and spread in reproducibility can be expected. It is obvious that the receptive fields vary in area extent from one type of cell to another. The important characteristic for visual acuity, we believe, is the response to small movements which, as is indicated as a depression in the center of the receptive field sensitivity profile and is strongest in the mid-periphery where the sensitivity of the excitation (ON) and the antagonistic inhibition (OFF) responses are approximately equal in magnitude. It is this type of balance that would be expected to be most sensitive to any changes in illumination and, thus, any movement of a discontinuity will stimulate the neural system. Often the inhibitory influences are the first to be affected by toxins and narcotics, almost any type of trauma to the neurons seems to weaken the inhibitory inputs. Thus, we would expect a disturbance of function of the neural elements in the retina would be manifested here earlier than at any other neural location. Exactly what will happen, whether the first sign will be diffuseness in



the visual detection response, or as we expect in the most severely affected cases, motion detection will almost disappear is unknown. Only experimental data will furnish the answer.

The injection of Tissue Plasminogen Activator (TPA) subretinally to test if it is a cause of receptor damage have been unsuccessful to date. No correlation as yet has been possible with associated projects by other contractors within this program. Hopefully when this information is obtained, it will furnish important guidance for the future therapy by the other investigators.

#### V. Suggested Follow-On Work in Future Periods Based This Program

Recordings from the area centralis of the cat retina can continue to furnish sensitive and reproducible information on the possible effects of all types of retinal trauma upon the receptive field organization as related to acuity. The effects on the various types of cone input, both in the center and surround, and particularly as regards to transient response should be the focus of the data analysis.

A mathematical model based on the motion detection by X and Y cells and related to the combination of the absolute values of the differentials of the Gaussian curves which describe the sensitivity profiles of the excitatory and inhibitory inputs should be investigated in more detail as it may be made the basis of an electronic type of discontinuity detector. Tests should be made to find the function that is used by the retina to see if it is the optimum one. Preliminary experiments done during the contract period to test this function have been inconclusive, and thus more should be done in the future.

The recordings from the cat retina should be continued to show the effects of exposures to the various types of lasers, from animals made available by a companion set of experiments in which the retina is inactivated by a subretinal hemorrhage, which is allowed to recover naturally or may be surgically removed. These retinas should be tested to learn the effects, if any, from such trauma to the receptor layer.

In summary, experiments in the future should be designed to give a comparison of the effects of subretinal hemorrhages and recovery on the retinal responses from animals that are available from other laser injury treatment programs.

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Figure 1

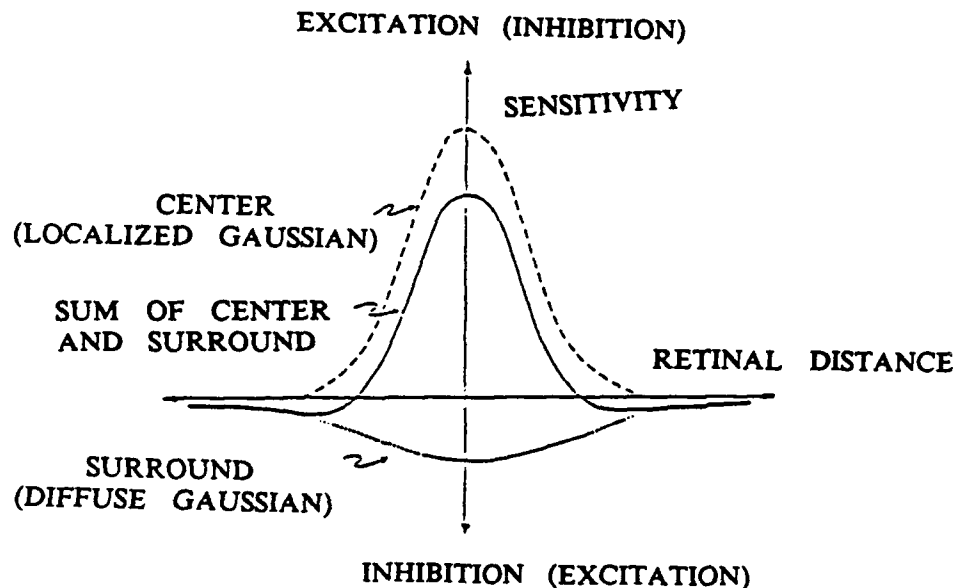


Figure 1. Receptive field sensitivity profile of a retinal ganglion cell to ON/OFF stationary retinal image stimulation. (Sensitivity is the inverse of the intensity needed to give a standard response). Receptor input reaches the ganglion cell through 2 input channels. The first (center) is a rather restricted central area of receptors having positive (or negative) input through the intermediate neuronal layers to the ganglion cell. This profile is Gaussian and may arise from more than one type of receptor. The second input (surround) has an opposing type of input in a more diffuse Gaussian. The solid curve is the summed input from a stimulus which activates both inputs (based on Wagner, *et al.*, 1963, and Rodieck, 1965).

Figure 2

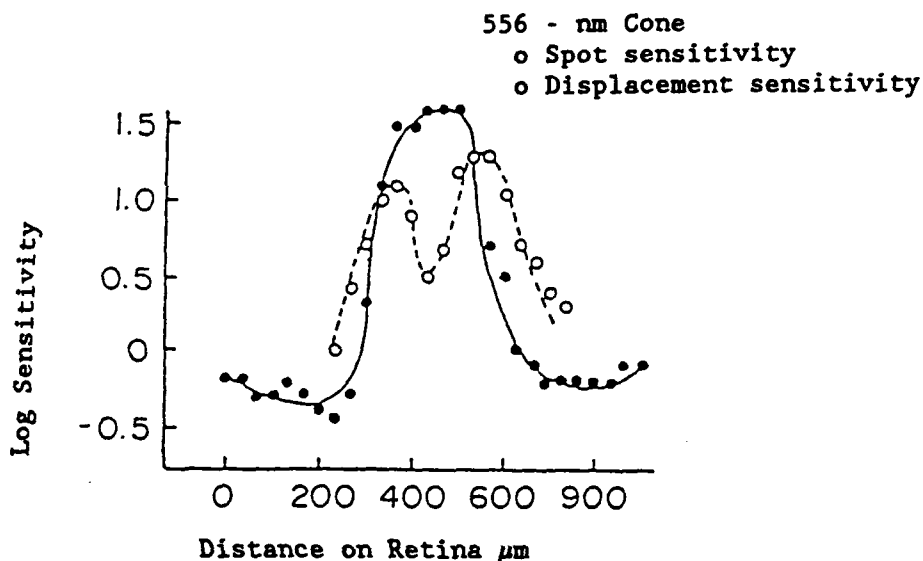


Figure 2. Static and displacement sensitivity of a ganglion cell. The spot ON sensitivity (filled circles) was measured with a  $35 \mu\text{m}$  (10 minutes of arc) spot of yellow (580 nm) light. This spot was positioned, then flashed at 0.5 HZ until a threshold was determined for that position. The static spot sensitivity was measured every  $35 \mu\text{m}$ . The blue background adapting light was provided by Wratten #47, equivalent for the rods to  $6.0 \times 10^{10}$  photons  $\text{deg}^{-2}$  of 500 nm light. The blue background assured that the cell responses were mediated by the 556 nm cone. Spot OFF sensitivity was measured with a  $35 \mu\text{m}$  (on the retina) spot jerk quickly (less than 0.1 sec) between two positions  $35 \mu\text{m}$  apart. Between each displacement the cell remained unstimulated for 1 second. Each displacement sensitivity is plotted halfway between the two end positions. The displacement sensitivity was measured every  $35 \mu\text{m}$  across the receptive field. The continuous and dashed curves were drawn in by the eye. Zero log units on the sensitivity axis is  $1.75 \times 10^{11}$  photons  $\text{deg}^{-2} \text{sec}^{-1}$  at the cornea, except that negative log sensitivity numbers are for the OFF response from the surround and should be read as absolute values.

Figure 3

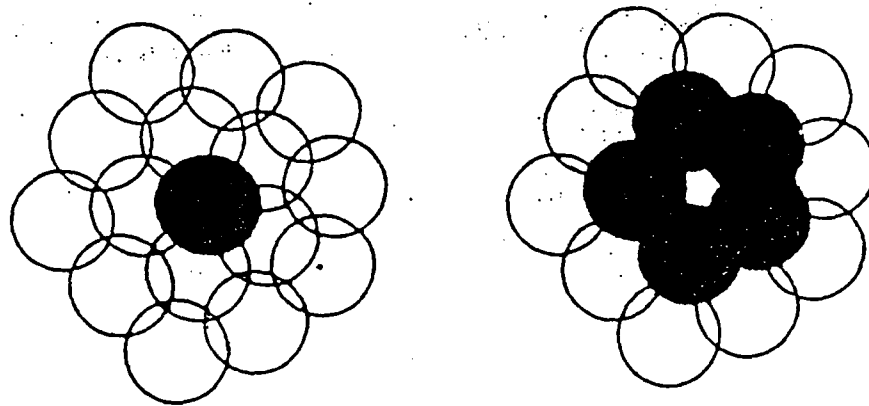


Figure 3. Multiple ganglion cell outputs to a small discontinuity. The left hand drawing represents the overlap of the detectors making up the central sensitivity zones as shown in figure 1. The dark circle represents the cell having maximum output when the intensity is changed suddenly. The right hand drawing represents the same overlapping central receptive fields of the same ganglion cells as on the left. The dark ring represents those ganglion cells responding maximally to a small displacement of a discontinuity in the center. These responses are from ganglion cells whose centers are eccentric to the location of discontinuity. The information as to the presence and the location of the discontinuity has a much better signal to noise ratio here than in the left hand figure as more ganglion cells respond and can be averaged. Also, as different ganglion cells have different contrast detection conditions, the detection of discontinuity can be accomplished regardless of the type of contrast, color or intensity present at that time. Because a large amount of averaging is possible, more certainty can be attained at lower contrast levels. The group or "cluster" of ganglion cells which respond will be connected to the next stage of neurons in similar types of overlapping receptive fields. As described in the text, the high frequency data corresponding to the discontinuity movement could be separated from lower frequency steady state component corresponding to the intensity or color data information, and placed in a separate layer (but in register) from that giving the eccentricity (cluster) response layer of neurons for further computation.

Figure 4

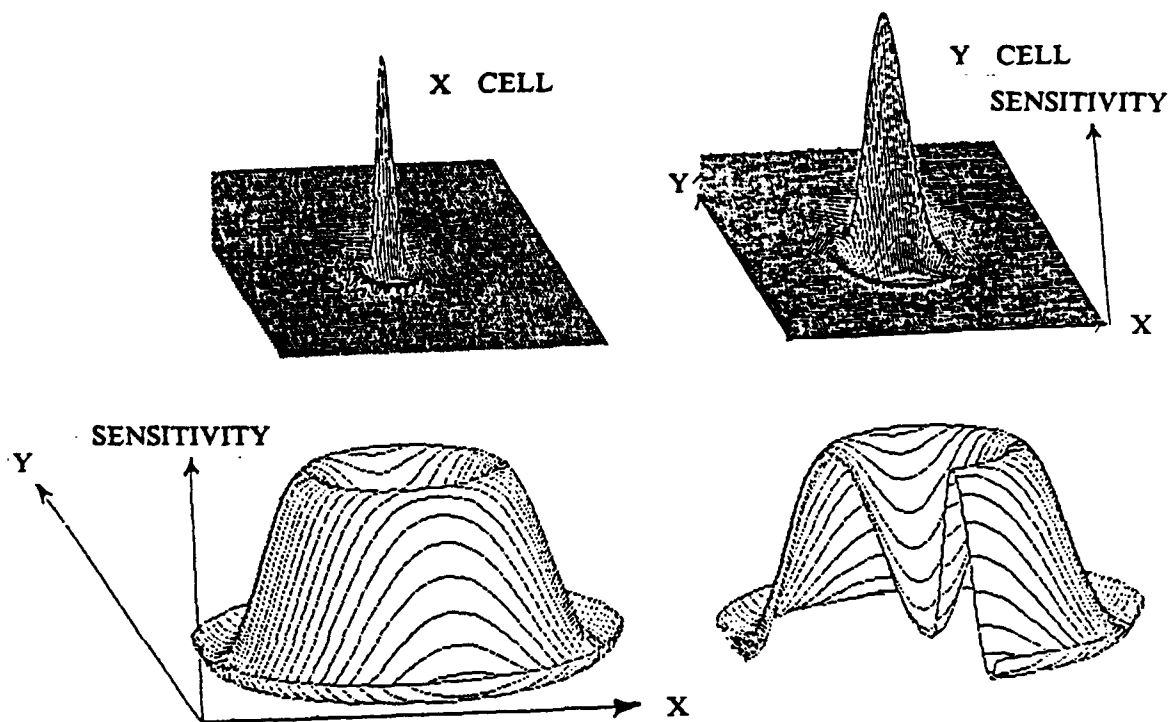
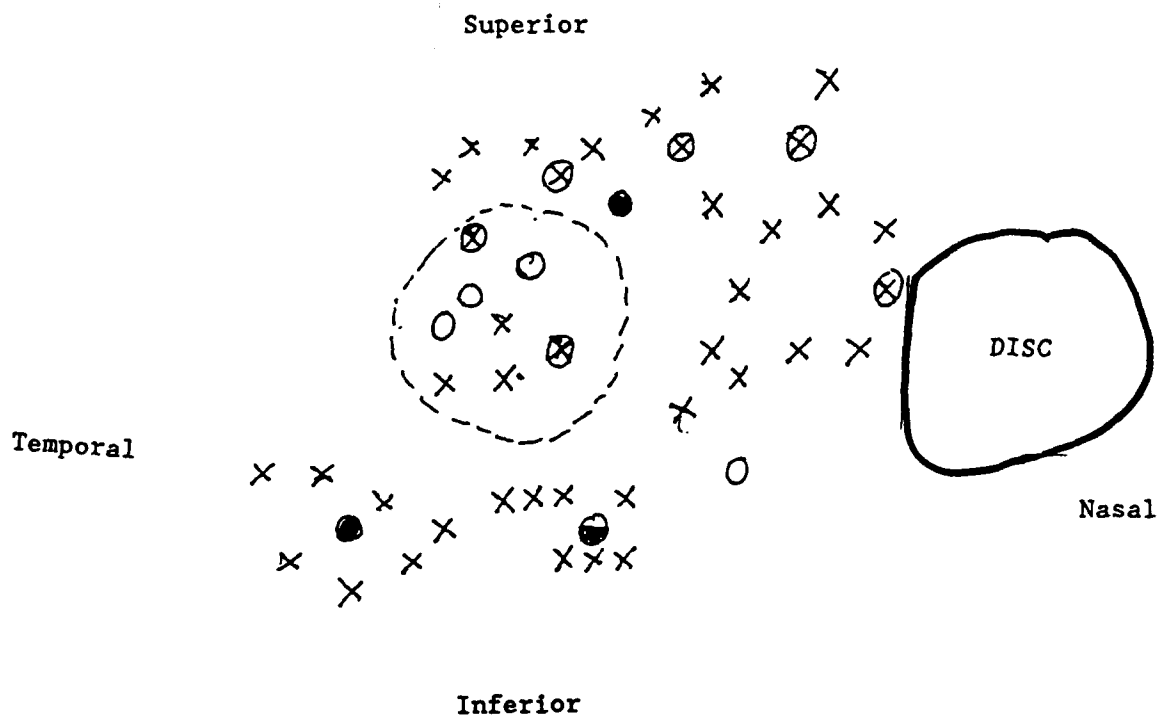


Figure 4. Three dimensional representation of the sensitivity profiles for retinal ganglion cells. The profiles shown are solids of rotation for the curves shown in Fig. 1 and 2. The upper plots are based on the summed curve (solid line) in Fig. 1, which itself is an idealized representation of the solid line defined by the filled circle data points in Fig. 2. The upper plots are the responses to be expected from stationary stimulus spots of relatively long duration (0.5 sec.). The lower plots represent the idealized forms of the dashed curve in Fig. 2 for a Y cell, and are for the responses to small, single, rapid movements of a constantly illuminated stimulus spot. The important feature is the dip in sensitivity at the center of the receptive field shown in the lower plots indicating that a moving stimulus will have the most vigorous response in an annulus around the center. See text for more extensive discussion.

Figure 5



X unsuccessful

- approximate location of the area centralis

● x cell

○ y cell

◐ w cell

○ unclassified cell

Figure 5. A diagram of the retina of the left eye of a cat. The types of ganglion cells recorded from are shown. The dashed line indicates the region of the area centralis. Circles are recordings that lasted 5 mins or more. The circle filling indicates the cell type. Unclassified cells were successful recordings whose responses did not fit into x or y categories very easily, but otherwise gave reproducible responses and were perhaps best described in a center surround antagonist scheme.

Figure 6

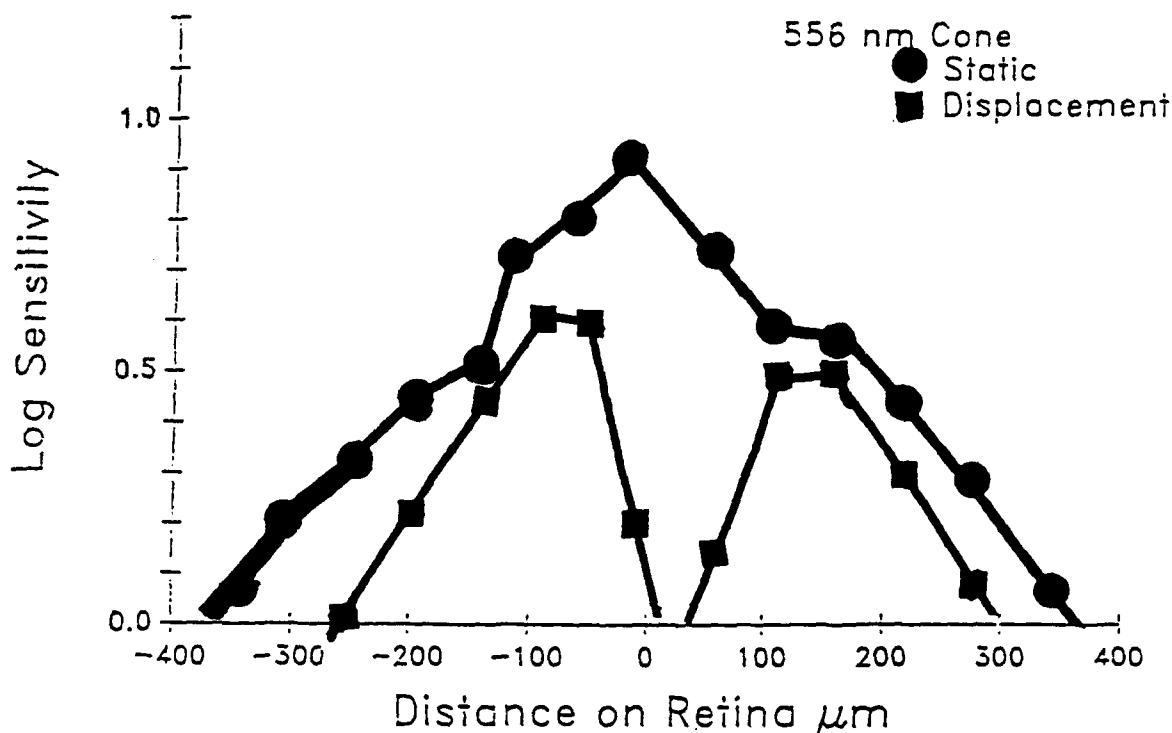


Figure 6. Static and displacement sensitivity profiles from a retinal ganglion cell. The circles are threshold responses to a stationary spot of 560 nm wavelength with a duration of 0.5 s recurring every 5 s. The squares are threshold responses to the same stimulus spot which is on continuously but moves its own diameter every 5 s in one rapid motion ( $\approx 1$  ms). The spot diameter is  $75 \mu\text{m}$  in the retinal plane. Sensitivity is the inverse function of retinal illuminance at the threshold response. The stimulus brightness could not be raised to a high enough level to elicit a threshold response in the central portion of the field, although a comparison with the data from other cells suggests that a factor of 3 more in brightness would have been sufficient. Zero log units on the sensitivity axis is  $1.75 \times 10^{11}$  photons  $\text{deg}^{-2} \text{s}^{-1}$ . Compare with figure 2 and consult text for further discussion.